causing a mutation in the selected skin gene which mutation leads to the skin disorder, wherein the skin disorder is an epidermal fragility disorder, a keratinization disorder or albinism disorder.

Remarks

Entry of this amendment and reconsideration of the subject application in view thereof are respectfully requested.

Applicant wishes to thank the Examiner for the telephone interview in this matter on March 4, 2002 with the Applicant's attorney, Nanda Kumar. During the interview, the pending claims and prior art rejections were discussed.

I. Claims

Claims 1-40 were pending in the application and these claims stood rejected.

Claims 1 and 32 have been amended to clearly define the invention. Applicant respectfully submits that no new matter is added by these amendments.

II. Rejections Under 35 U.S.C. § 112 Second Paragraph

Claims 1-17 stood rejected under 35 U.S.C. § 112, second paragraph as indefinite. Applicant has amended claim 1 to reflect language corresponding to that used in claim 18 as suggested by the Examiner on page 5 of the Office Action. Applicant believes that this clarifying amendment obviates the asserted ground for the rejection. Reconsideration is respectfully requested.

III. Rejections Under 35 U.S.C. §102

A. Alexeev

Claims 32-34 and 37-39 stood rejected as allegedly being anticipated by Alexeev et al., 1998, Nature Biotechnology 16:1343-1346. Applicant respectfully traverses this rejection.

Alexeev et al. is cited for teaching the claimed product though not by the same process. Applicant respectfully disagrees and submits that this reference teaches a method of correcting a

mutation in skin cells *in vitro* by an RND-DNA oligonucleotide but does not teach an animal model having a skin disorder. Therefore, Alexeev does not anticipate the rejected claims. Accordingly, withdrawal of this rejection is respectfully requested.

B. Christiano et al

Claims 32-33, 35 and 37-39 stood rejected as allegedly being anticipated by Christiano et al., 1994, Proc. Natl. Acad. Sci, 91:3549-3553. Applicant respectfully traverses this rejection.

The Examiner cites Christiano for teaching Finnish individuals with a mutation in the COL7A1 gene and avers that these animals represent the claimed animal model though not produced by the same process. Claim 32 as amended is directed a non-human animal model. Christiano does not teach a non-human animal model with a mutation in a gene in skin cells and, therefore, does not anticipate the rejected claims. Withdrawal of this rejection is respectfully requested.

C. Uttam et al.

Claims 32-33 and 36-39 stood rejected as allegedly being anticipated by Uttam et al., 1996, Proc. Natl. Acad. Sci, 93:9079-9084. Applicant respectfully traverses this rejection.

The Examiner cites Uttam for teaching German individuals with a mutation in the keratin 14 gene and avers that these animals represents the claimed animal model though not produced by the same process. Claim 32 as amended is directed to a non-human animal model. Uttam does not teach a non-human animal model with a mutation in a gene in skin cells and, therefore, does not anticipate the rejected claims. Withdrawal of this rejection is respectfully requested..

IV. Rejections Under 35 U.S.C. §103

Claims 1-40 stood rejected as allegedly being obvious over Yoon et al., 1996, Proc. Natl. Acad. Sci., 93:2071-2076 and Alexeev et al., 1998, Nature Biotechnology 16:1343-1346, in view of Uttam et al., 1996, Proc. Natl. Acad. Sci, 93:9079-9084, Christiano et al., 1994, Proc. Natl. Acad. Sci, 91:3549-3553 and Cole-Strauss et al., 1996, Science, 273:1386-1389. Applicant respectfully traverses this rejection.

Yoon et al. teaches modifications of a gene on a plasmid in chinese hamster ovary cells (host cells) *in vitro*. The Examiner points to the abstract of this reference. The abstract states

that [t]hese results extend the usefulness of the oligonucleotide-based gene targeting approaches by increasing specific targeting frequency. This strategy should enable the design of antiviral agents." Thus, the disclosure of the Yoon reference is relevant to modification of episomal genes or viral genes. The present invention does not deal with modification of episomal genes or viral genes at all, but rather deals with genetic modifications in skin cells *in vivo*, something that Yoon et al. does not even mention. Applicant respectfully points out that intact organs such as skin have several layers of cells and are made up of more than one type of cells (e.g., keratinocytes, melanocytes, fibroblasts etc.,). For the details on the complexity of skin structure, see, for example Haake et al., 1999, The structure and development of skin, Chapter 7, in: Fitzpatrick's Dermatology in General Medicine, Freedberg et al., eds, McGraw-Hill (pertinent pages of this article are enclosed). While Yoon teaches the modification of episomal genes *in vitro*, this reference does not teach or suggest gene modifications in an organ such as skin. In contrast to an *in vivo* situation, the cells cultured *in vitro* can easily be manipulated to introduce genes and modification of genes can be easily achieved because of highly controlled environment and a direct access to the target cells and genes therein. Stated otherwise, the accomplishment of the modification of episomal genes *in vitro* cannot be a ground for the rejection of claims directed to modification of genes *in vivo* given the lack of correlation to an *in vivo* situation and hence the lack of a reasonable expectation of success.

Like Yoon et al., Alexeev teaches genetic modifications in skin cells *in vitro* not *in vivo*. Alexeev's *in vitro* approach uses melan-c cell and does not involve the intact skin and its complexity. The accomplishment of the modification of genes in melan-c cells *in vitro* cannot be a ground for the rejection of claims directed to modification of skin genes *in vivo* given the lack of correlation to an *in vivo* situation and hence the lack of a reasonable expectation of success.

Cole-Strauss teaches correction of a mutation in β-globin genes of lymphoblastoid cell *in vitro*. There is no teaching or suggestion of gene modifications of skin cell genes either *in vitro* or *in vivo*. Uttam and Christiano references simply report mutations in skin genes. These references do not teach, suggest or contemplate genetic modifications in skin cells by RDO approach.

A determination of obviousness must involve more than indiscriminate combination of the prior art; a suggestion or motivation to combine must exist. *Micro Chemical, Inc. v. Great Plains Chemical Co., Inc.*, 103 F.3d 1538, 41 USPQ2d 1238 (Fed. Cir. 1997), *cert. denied*, 117 S.Ct. 2516 (1997). None of the cited references speak to the issues related to gene modifications in cells of a mammalian skin *in vivo,* much less suggests how this can be done. Applicant notes that Yoon, Alexeev and Cole-Strauss references use chimeric RNA-DNA oligonucleotides to modify genes *in vitro*, and teach modification of different genes, episomal or nuclear, in different cell types under different conditions with no guidance, whatsoever, on how to successfully modify genes in skin cells in an *in vivo* situation. Uttam and Christiano references do not cure the deficiencies in Yoon, Alexeev and Cole-Strauss references. In essence, there are no teachings in the cited art to suggest the desirability, and thus obviousness, of combining these references in a way that would lead to the claimed method. There is no reasonable expectation of success. Applicant respectfully submits that the cited combination of references is nothing more than an indiscriminate combination of prior art references in an attempt to reconstruct the claimed invention by hindsight.

It may also be noted that Alexeev relates to gene modifications in cultured skin cells (*in vitro*) not in an intact skin *in vivo.* Alexeev's *in vitro* method resulted in gene modifications with a frequency range of only 0.01-15%. While it may be argued, in light of Alexeev reference, that it would have been obvious to one skilled in the art to try [1]Alexeev's method, in an *in vivo* context, it would nevertheless be nonobvious to one of ordinary skill in the art that such a modification of Alexeev would result in the unexpectedly high frequency of gene modifications (approaching 40%) in cells of a mammalian skin *in vivo* as found by the Applicant. Applicant's *in vivo* results were greater than those which would have been expected from Alexeev's limited *in vitro* successes, ranging from almost zero to 15%. In fact, one would expect that Alexeev would have achieved a higher frequency of gene correction in vitro than in

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[1] Obvious to try is an improper ground for a §103 rejection. *In re Dow Chemical Co.* 5 USPQ2d 1529 (Fed Cir 1988)

vivo, exactly the opposite of what the applicant has achieved. Such an unexpected result, which is of a significant practical advantage, is an indication of nonobviousness. *In re Soni,* 34 USPQ2d 1684 (Fed. Cir. 1995).

Accordingly, Applicant respectfully requests that the cited references fail to meet the requirements for obviousness under 35 U.S.C. § 103(a). Reconsideration and withdrawal of the rejection are respectfully requested.

V. Conclusion

A favorable reconsideration in view of the above remarks and allowance of the pending claims in the application are earnestly solicited.

Respectfully submitted,

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William J. McNichol, Jr.
Registration No. 31,179
Nanda P.B.A. Kumar
Registration No. 44,853
Attorneys for Applicant

REED SMITH LLP
2500 One Liberty Place
1650 Market Street
Philadelphia, Pennsylvania 19103-7301
Fax: (215) 241-7945
Attn: William J. McNichol, Jr., Esq.
(215 241-7950)
Nanda P.B.A. Kumar, Esq.
(215 241-7991)

Marked Up Version of Claims in Serial No. 09/473,872 in response to the Office Action of December 6, 2001

1. (Thrice Amended) A method of modifying a selected gene in cells of a human skin in vivo which comprises delivering to said cells at one or more locations of the human skin an effective amount of a composition [sufficient to bring about stable genetic and phenotypic modifications in the selected gene at said locations wherein the composition comprises] comprising a chimeric RNA-DNA oligonucleotide having a double hairpin structure with pyrimidine loops and a pharmaceutically acceptable carrier such that [said] stable genetic modifications are made to the selected gene which result in phenotypic changes at said locations of the human skin.

32. (Twice Amended) [An] A non-human animal model having a skin disorder at one or more locations of its skin wherein the skin disorder is a result of a treatment at said locations with a composition comprising a chimeric RNA-DNA oligonucleotide having a double hairpin structure with pyrimidine loops targeted to a selected skin gene, said oligonucleotide thereby causing a mutation in the selected skin gene which mutation leads to the skin disorder, wherein the skin disorder is an epidermal fragility disorder, a keratinization disorder or albinism disorder.

APPENDIX: Pending claims in application Serial No. 09/473,872 after the entry of the amendment filed on April 26, 2002 in response to the Office Action dated December 6, 2001:

1. (Thrice Amended) A method of modifying a selected gene in cells of a human skin in vivo which comprises delivering to said cells at one or more locations of the human skin an effective amount of a composition comprising a chimeric RNA-DNA oligonucleotide having a double hairpin structure with pyrimidine loops and a pharmaceutically acceptable carrier such that stable genetic modifications are made to the selected gene which result in phenotypic changes at said locations of the human skin.

2. The method of claim 1, wherein the stable genetic modification is in an epidermal fragility disorder gene selected from the group consisting of COL7A1, LAMA3, LAMB3, LAMC2, COL17A1, ITGA6, ITGB4, PLEC1, KRT5, KRT14 and PKP1.

3. The method of claim 1, wherein the stable genetic modification is in a keratinization disorder gene selected from the group consisting of KRT1, KRT10, KRT9, KRT16, LOR, KRT2e, KRT6a, KRT 16, KRT 17, STS, TGM1, GJB2, GJB3, ATP2A2, DSP and DSG1.

4. The method of claim 1, wherein the selected gene is tyrosinase, COL7A1, LAMA3, LAMB3, LAMC2, COL17A1, ITGA6, ITGB4, PLEC1, KRT5, KRT14, PKP1, KRT1, KRT10, KRT9, KRT16, LOR, KRT2e, KRT6a, KRT 16, KRT 17, STS, TGM1, GJB2, GJB3, ATP2A2, DSP, DSG1, HR, hHB1, hHB6, PAX3, TYR, TYRP-1, OCA2, OA1, MITF, HPS, FECH, UROS, URO-D, XPA, XPB, XPC, XPD, XPG, PTC, STK11/LKB1, PTEN, PTEN, XPB, XPD, WHN, GLA, ATM, ENG, ALK-1, PPO, BPAG2, or DSG3 gene.

5. The method of claim 1, wherein the selected gene is tyrosinase gene.

6. The method of claim 1, wherein the selected gene is COL7A1 gene.

7. The method of claim 1, wherein the selected gene is KRT17 gene.

8. The method of claim 1, wherein the chimeric RNA-DNA oligonucleotide comprises:

(a) a first string of nucleotides wherein the first string is made of at least four contiguous deoxyribonucleotides flanked on each side by at least nine ribonucleotides; and

(b) a second string of deoxyribonucleotides that is fully complementary to the first string of nucleotides, and

wherein the chimeric RNA-DNA oligonucleotide is, RNase protected, and wherein the chimeric RNA-DNA oligonucleotide has in each of the first and second strings a set of contiguous nucleotides that are fully complementary to a segment of DNA of the selected gene except that the first string has one mismatching deoxyribonucleotide in said contiguous deoxyribonucleotides that defines a site of modification in the selected gene.

9. The method of claim 1, wherein the chimeric RNA-DNA oligonucleotide comprises:

(a) a first string of nucleotides wherein the first string is made of at least 20 ribonucleotides; and

(b) a second string of deoxyribonucleotides having the same number of deoxyribonucleotides as in the first string of nucleotides, wherein the second string is fully complementary to the first string of nucleotides except that the second string has a deoxyribonucleotide that forms a mismatched base pair with the corresponding nucleotide in the first string, and

wherein the chimeric RNA-DNA oligonucleotide is RNase protected, and wherein the chimeric RNA-DNA oligonucleotide has in each of the first and second strings a set of contiguous nucleotides that are fully complementary to a segment of DNA of the selected gene except that the deoxyribonucleotide in the second string also forms a mismatched base pair with

the corresponding deoxyribonucleotide in the DNA strand of the selected gene which mismatched base pair defines a site of modification in the selected gene.

10. The method of claim 1, wherein the chimeric RNA-DNA oligonucleotide comprises:

(a) a first string of nucleotides wherein the first string is made of at least four contiguous deoxyribonucleotides flanked on each side by at least nine ribonucleotides; and

(b) a second string of deoxyribonucleotides that is fully complementary to the first string of nucleotides, and

wherein the chimeric RNA-DNA oligonucleotide is RNase protected, and wherein the chimeric RNA-DNA oligonucleotide has in each of the first and second strings a set of contiguous nucleotides that are fully complementary to a segment of DNA of the selected gene except that the first and second strings have one, two or four pairs of nucleotide insertions or deletions that defines a site of modification in the selected gene.

11. The method of claim 1, wherein the stable genetic modification is correction of a mutation.

12. The method of claim 11, wherein the mutation is a point mutation or a frame shift mutation.

13. The method of claim 1, wherein the stable genetic modification is generation of a mutation.

14. The method of claim 13, wherein the mutation is a point mutation or a frame shift mutation.

15. The method of claim 13, wherein the mutation is a dominant mutation.

16. The method of claim 1, wherein said phenotypic changes include the correction of a skin disorder.

17. The method of claim 1, wherein said phenotypic changes include the correction of albinism, an epidermal fragility disorder or a keratinization disorder.

18. A method of modifying a selected gene in cells of an animal skin in vivo which comprises delivering to said cells at one or more locations of the animal skin an effective amount of a composition comprising a chimeric RNA-DNA oligonucleotide having a double hairpin structure with pyrimidine loops and a pharmaceutically acceptable carrier such that the stable genetic modifications are made to the selected gene which result in phenotypic changes at said locations of the animal skin, wherein the animal is a mouse.

19. The method of claim 18, wherein the selected gene is tyrosinase, COL7A1, LAMA3, LAMB3, LAMC2, COL17A1, ITGA6, ITGB4, PLEC1, KRT5, KRT14, PKP1, KRT1, KRT10, KRT9, KRT16, LOR, KRT2, KRT6, KRT 16, KRT 17, STS, TGM1, GJB2, GJB3, ATP2A2, DSP, DSG1, HR, hHB1, hHB6, PAX3, TYR, TYRP-1, OCA2, OA1, MITF, HPS, FECH, UROS, URO-D, PPO, XPA, XPB, XPC, XPD, XPG, PTC, STK11/LKB1, PTEN, PTEN, XPB, XPD, WHN, GLA, ATM, ENG, ALK-1, a cytokine BPAG2 or DSG3 gene.

20. The method of claim 18, wherein the selected gene is tyrosinase gene.

21. The method of claim 18, wherein the selected gene is COL7A1 gene.

22. The method of claim 18, wherein the selected gene is KRT17 gene.

23. The method of claim 18, wherein the chimeric RNA-DNA oligonucleotide comprises:

(a) a first string of nucleotides wherein the first string is made of at least four contiguous deoxyribonucleotides flanked on each side by at least nine ribonucleotides; and

(b) a second string of deoxyribonucleotides that is fully complementary to the first string of nucleotides, and

wherein the chimeric RNA-DNA oligonucleotide is RNase protected, and wherein the chimeric RNA-DNA oligonucleotide has in each of the first and second strings a set of contiguous nucleotides that are fully complementary to a segment of DNA of the selected gene except that the first string has one mismatching deoxyribonucleotide in said contiguous deoxyribonucleotides that defines a site of modification in the selected gene.

24. The method of claim 18, wherein the chimeric RNA-DNA oligonucleotide comprises:

(a) a first string of nucleotides wherein the first string is made of at least 20 ribonucleotides; and

(b) a second string of deoxyribonucleotides having the same number of deoxyribonucleotides as in the first string of nucleotides, wherein the second string is fully complementary to the first string of nucleotides except that the second string has a deoxyribonucleotide that forms a mismatched base pair with the corresponding nucleotide in the first string to make the genetic modifications in the selected gene, and

wherein the chimeric RNA-DNA oligonucleotide is RNase protected, and wherein the chimeric RNA-DNA oligonucleotide has in each of the first and second strings a set of contiguous nucleotides that are fully complementary to a segment of DNA of the selected gene except that the deoxyribonucleotide in the second string also forms a mismatched base pair with the corresponding deoxyribonucleotide in the DNA strand of the selected gene which mismatched base pair defines a site of modification in the selected gene.

25. The method of claim 18, wherein the chimeric RNA-DNA oligonucleotide comprises:

(a) a first string of nucleotides wherein the first string is made of at least four contiguous deoxyribonucleotides flanked on each side by at least nine ribonucleotides; and

(b) a second string of deoxynribonucleotides that is fully complementary to the first string of nucleotides, and

wherein the chimeric RNA-DNA oligonucleotide is RNase protected, and wherein the chimeric RNA-DNA oligonucleotide has in each of the first and second strings a set of contiguous nucleotides that are fully complementary to a segment of DNA of the selected gene except that the first and second strings have one, two or four pairs of nucleotide insertions or deletions that defines a site of modification in the selected gene.

26. The method of claim 18, wherein the stable genetic modification is correction of a mutation.

27. The method of claim 26, wherein the mutation is a point mutation or a frame shift mutation.

28. The method of claim 18, wherein the stable genetic modification is generation of a mutation.

29. The method of claim 28, wherein the mutation is a point mutation or a frame shift mutation.

30. The method of claim 28, wherein the mutation is a dominant mutation.

31. The method of claim 18, wherein said phenotypic changes include the correction of albinism, an epidermal fragility disorder or a keratinization disorder.

32. (Twice Amended) A non-human animal model having a skin disorder at one or more locations of its skin wherein the skin disorder is a result of a treatment at said locations

with a composition comprising a chimeric RNA-DNA oligonucleotide having a double hairpin structure with pyrimidine loops targeted to a selected skin gene, said oligonucleotide thereby causing a mutation in the selected skin gene which mutation leads to the skin disorder, wherein the skin disorder is an epidermal fragility disorder, a keratinization disorder or albinism disorder.

33. The animal model of claim 32, wherein the selected skin gene is Tyr, COL7A1, LAMA3, LAMB3, LAMC2, COL17A1, ITGA6, ITGB4, PLEC1, KRT5, KRT14, PKP1, KRT1, KRT10, KRT9, KRT16, LOR, 1998, KRT2e, KRT6a, KRT 16, KRT 17, STS, TGM1, GJB2, GJB3, ATP2A2, DSP, DSG1, HR, hHB1, hHB6, PAX3, TYR, TYRP-1, OCA2, OA1, MITF, HPS, FECH, UROS, URO-D, PPO, XPA, XPB, XPC, XPD, XPG, PTC, STK11/LKB1, PTEN, PTEN, XPB, XPD, WHN, GLA, ATM, ENG, ALK-1, a cytokine BPAG2 or DSG3 gene.

34. The animal model of claim 33, wherein the selected gene is Tyr gene.

35. The animal model of claim 33, wherein the selected gene is COL7A1 gene.

36. The animal model of claim 33, wherein the selected gene is KRT17 gene.

37. The animal model of claim 32, wherein the skin disorder is due to generation of a mutation in the selected skin gene.

38. The animal model of claim 37, wherein the mutation is a point mutation or a frame shift mutation.

39. The animal model of claim 37, wherein the mutation is a dominant mutation.

40. A method of correcting a mutation in a tyrosinase gene in cells of a mammalian skin in vivo which comprises delivering to said cells at one or more locations of the mammalian skin an effective amount of a composition comprising a Tyr-A RNA-DNA oligonucleotide for

causing stable genetic correction in the tyrosinase gene and a pharmaceutically acceptable carrier such that the correction results in restoration of tyrosinase enzyme activity at said locations of the mammalian skin, wherein the mammalian skin is selected from the group consisting of a human and a mouse.